

CHALKLEY, HAROLD WILLIAM

Changes in Water Content in *Amoeba*
in Relation to Changes in Its
Protoplasmic Structure

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CHANGES IN WATER CONTENT IN AMOEBA IN RELATION TO CHANGES IN ITS PROTOPLASMIC STRUCTURE¹

(Sixteen figures)

H. W. CHALKLEY

Zoölogical Laboratory, Johns Hopkins University

INTRODUCTION

LILLIE (1909) asserts that stimulation of irritable cells is dependent upon changes in permeability, and that changes in permeability are dependent upon changes in surface and interprotoplasmic films.

Verworn (1892), Chambers (1920), Bayliss (1921), Edwards (1921), Schwittalla (1921), Folger (1923), Mast (1923 and 1926), Hopkins (1926), and others account for various different physical effects produced in *Amoeba* by environmental factors, by assuming that these factors produce changes, either local or general, in "solation" or "gelation" or "fluidity"; thus implying that these produce changes in the colloid structure of the protoplasm.

Mast (1926a) asserts that the main cell-mass in *Amoeba* is composed of two layers, an inner more fluid layer, the "plasmasol" and an outer more rigid layer the "plasmagel." He maintains that these components differ mainly in the presence within the plasmagel of a "relatively solid framework," to which is due the rigidity of the plasmagel. This framework he asserts is probably composed of interrelated layers, i.e., it is an interprotoplasmic film structure. He describes minutely (1926b) the continuous transformation of the plasmasol to plasmagel, and vice versa, that forms one of the characteristic phenomena of locomotor activity in *Amoeba*, calls attention to the variation in thickness of the plasmagel with changes in external conditions, and contends that all phenomena of "solation" or "gelation" or changes in "fluidity" are correlated with local or general formation or dissolution of the film structure in the plasmagel.

¹ This article is a revision of a dissertation presented, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, to the Board of University Studies of the Johns Hopkins University.

If these contentions are true, this film structure in *Amoeba*, and probably a similar structure in other cells, plays a rôle of considerable importance, and is correlated in its changes with changes in activity whether of locomotion, response to stimulus, regulation of water content, or other kindred phenomena.

Indirect evidence of this is not wanting, for every observation of change in the rigidity of the cell, however induced, may be thus interpreted. Evidence of this type, however, does not constitute proof of a relation between the state of the interprotoplasmic film structure and an external factor. It offers no opportunity for differentiation between change in rigidity involving the entire content of the cell, such as might be produced by hydration or dehydration of the entire protoplasm, and change in rigidity due to increase or decrease in the development of the interprotoplasmic film structure, which might conceivably occur with little reference to viscosity changes in the other cell constituents.

The experiments presented here were undertaken for the purpose of ascertaining whether in fact such a relation exists. They constitute an exploratory investigation into the general relation existing between the water content of *Amoeba*, as indicated by changes in volume, and the balance between the liquefying and solidifying tendencies in its cytoplasm, as shown by changes in the volumetric ratio between the more solid, film-supported plasmagel and the more liquid plasmasol.

For the convenience of the reader, the experiments on water content and on the changes in the plasmagel-plasmasol ratio are presented separately. Those dealing with the water content are presented first.

MATERIAL

The amoebae used conformed closely to the type *Amoeba proteus* (Schaeffer, 1916). They were cultured in three different media, in finger bowls: *A*, hay infusion made with half spring and half distilled water; *B* hay infusion made with modified Ringer solution¹ (Hopkins, 1926) diluted to one-thirtieth, and buffered to

¹ The modified Ringer solution was made up as follows: 0.35 gram NaCl, 0.14 gram KCl, 0.12 gram CaCl₂, redistilled water to make one liter of solution. In using this, 3.3 cc. of the described solution were taken to which were added 5 cc. of phosphate

pH 6.8; C, hay infusion made with modified Ringer solution diluted to one-sixtieth and buffered to pH 6.8. In these infusions the stems of timothy hay were used and were sterilized by fractional sterilization in the solutions used prior to adding buffer. The finger bowls contained about 200 cc. of solution each. The hydrogen-ion concentration in media A and B, which normally became alkaline with time, was maintained at the value desired by the removal when required of from 5 to 10 cc. of the media and substitution of a like amount of fresh infusion. In C, in which the tendency was to become acid, calcium carbonate, which had been found beneficial in unbuffered acid cultures was added from time to time until the C_H was restored to its initial value.

METHODS

Two methods were used for ascertaining the volume of *Amoeba*. They will be referred to as the "indirect" and the "direct" methods.

Indirect method.—The indirect method may be described as follows: *Amoeba* when active varies continuously in length and thickness, and tends to become spherical from time to time. When an *amoeba* is spherical the volume, of course, can be readily calculated, but the rarity of this occurrence makes such calculations of little use for experimental purposes. The diameter that an amoeba would have as a sphere can however be approximately ascertained, even if it does not actually become spherical, on the assumption that it is at all times comparable in form to an ellipsoid of rotation.

If the long axis of such an ellipsoid be varied in length, the volume remaining constant, it is obvious that as it increases the short axis must decrease and vice versa, and further that if the long axis decreases until it is equal to the short the ellipsoid becomes a sphere.

It is easily seen that given such an ellipsoid the axes of which fluctuate, but which at no time actually becomes a sphere, it is possible to secure a series of corresponding values for these axes, and, by plotting the values, to produce a graph which can be extended by extrapolation to the point at which these axes become equal to

buffer (made with KOH instead of the usual NaOH) and sufficient distilled water to make 100 cc. This is the $\frac{1}{30}$ solution, the $\frac{1}{60}$ solution is this diluted to 200 cc. of solution.

each other, and to the diameter of a sphere equal in volume to the ellipsoid.

Now, since *Amoeba* is roughly comparable to an ellipsoid of rotation, and varies greatly in length and in thickness, it is evident that, if appropriate measurements can be obtained, a corresponding series of axial values can be derived which by plotting can be used to ascertain the diameter of a sphere approximately equal in volume to the amoeba.

To obtain the required measurements of these axes of *Amoeba*, an apparatus, designated the "optical apparatus" (Fig. 1) was constructed. This consists essentially of an ordinary compound microscope which has in addition to the usual vertical objective an objective placed horizontally, and an arrangement of three prisms which reflect the image formed by the horizontal objective so that it and the image formed by the vertical objective can be viewed simultaneously by means of a single ocular. To facilitate observation with this apparatus a cell was constructed from thin slips of glass (Fig. 2).

In securing axial measurements on *Amoeba*, this apparatus was employed as follows: Using a Greenough binocular microscope and a capillary pipet, a single amoeba was selected and put into the cell together with sufficient fluid, and covered with a cover glass. The whole was placed upon the stage of the optical apparatus, and both objectives focused on the organism. There then appeared in the microscope two adjacent images which were simultaneous projections of the amoeba as viewed from above and from the side. By means of a camera lucida, outline drawings of both images were made at frequent intervals, until a series of thirty or more was secured.

Later it was found that the number could be reduced in special cases. Special care was taken to obtain in every series a great variation in length (long axis).

From the series of outline drawings thus secured the long and the short axes presented by the amoeba in each of the different forms assumed, as presented in each of the different pairs of drawings, were obtained as follows: The long axes were secured from the drawings by direct measurement to the nearest 0.5 mm. Since the two outlines of a pair were made almost simultaneously this axis was

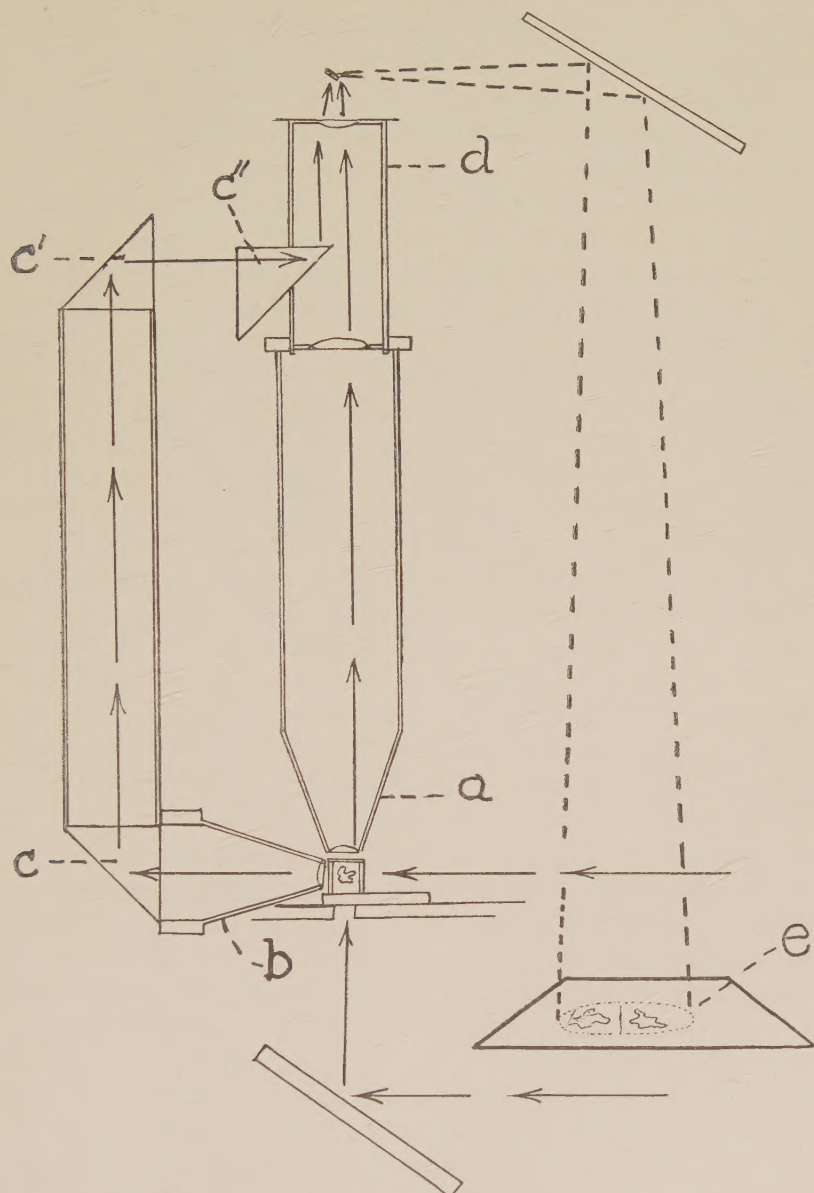


FIG. 1.—Diagram of optical apparatus used in making measurements of volume in *Amoeba*: *a*, vertical objective of microscope; *b*, horizontal objective; *c*, *c'*, *c''*, prisms reflecting image from horizontal objective into eyepiece; *d*, eyepiece receiving images from both vertical and horizontal objectives; *e*, images as projected by camera lucida.

common to both. The short axes were ascertained by measuring the area of each outline of each pair, dividing each of these areas by its corresponding length, computing the geometric means between the average heights and the average widths thus obtained, and then dividing each of these last values by one-quarter Pi.

This somewhat complicated method of obtaining the short axes was used to overcome the obstacles presented by the great variation in form in *Amoeba*. This variation is expressed in the drawings as irregularity of the perimeter due to laterally and vertically extended

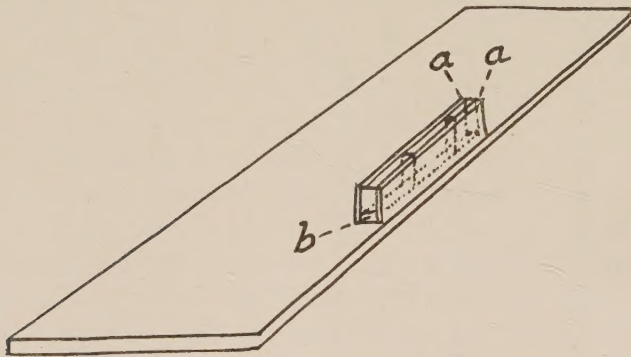


FIG. 2.—Cell used in conjunction with optical apparatus in making measurements of volume in *Amoeba*: *a, a*, glass sides; *b*, glass bottom; both bottom and sides are of polished glass thus permitting vision both from above and from the side.

pseudopodia. It produces such loose correlation between height, width, and length that neither height nor width, nor a mean between them, can be used as the short axis, for the basic assumption of the method requires that the linear characteristic of *Amoeba* used as this axis should vary inversely with the length, and become equal to the length only when *Amoeba* is spherical. Height or width alone will not fulfil either of these two requirements. A mean between them will partially fulfil the first but will not fulfil the last. Preliminary experimental measurements of outline drawings showed, however, that the geometrical mean between the average height and the average width of *Amoeba* does vary very nearly inversely as the long axis. This mean is equal to the long axis multiplied by one-quarter Pi, when the amoeba is spherical, and only then. It

therefore seemed that this mean divided by one-quarter π fulfils the requirements, and it was consequently adopted.

The areas were measured with an Ott planimeter to the nearest tenth of a square millimeter, and the lengths by scale to the nearest half millimeter.

After the axes for each pair of outlines had been measured they were plotted with the long axes as abscissas and the short as ordinates, and a smooth curve fitted to the points as described presently. This curve was then extended by extrapolation to the point where the axes would become equal, after which the volume was calculated by means of the usual formula $V = \frac{4}{3}\pi r^3$.

To test the accuracy of this method two experiments were performed, one with "plasticine" and the other with an amoeba.

Experiment 1.—A mass of plasticine was molded into a rough representation of an amoeba, laid upon a sheet of paper under a source of artificial light, and its shadow on the paper outlined with pencil. It was then turned on its long axis through 90 degrees and a second outline drawn. After this the plasticine was remolded to another form and the shadows were again projected and drawn. These processes were repeated until a series of eighteen or twenty pairs of outlines was made (Fig. 3). Axial measurements were then made from these drawings as described in the foregoing, and plotted, and then a curve was fitted to the plotted points as follows:

Trial showed that if the logarithms of the values found for the axes were plotted, instead of the actual values, the resulting graph approximated a straight line of negative slope. Hence, the relation between the axes is expressed by the formula $\log y = \log a - b \log x$, where y and x are any corresponding values for the long and short axes, respectively, and a and b are constants. The curve sought, therefore, has the formula $y = ax^{-b}$. This relation was found to hold in all graphs of measurements made on plasticine masses. The constants in the formula were evaluated from the logarithms of the axial measurements by the method of averages (Lipka, 1918, p. 126). Then, by substituting a series of values for y in the equation for the curve and solving for x a series of calculated axial values was obtained, including the value for the axes at equality. These were plotted on the graph and a curve drawn through them for com-

parison with the experimentally determined values and, finally, from the diameter of the sphere equivalent in volume to the mass, as indicated by the value calculated for the point where the axes would become equal, the volume was calculated in the usual way.

In this way, the volumes of four different masses of plasticine were calculated. Then, as a check on the calculations, the volumes

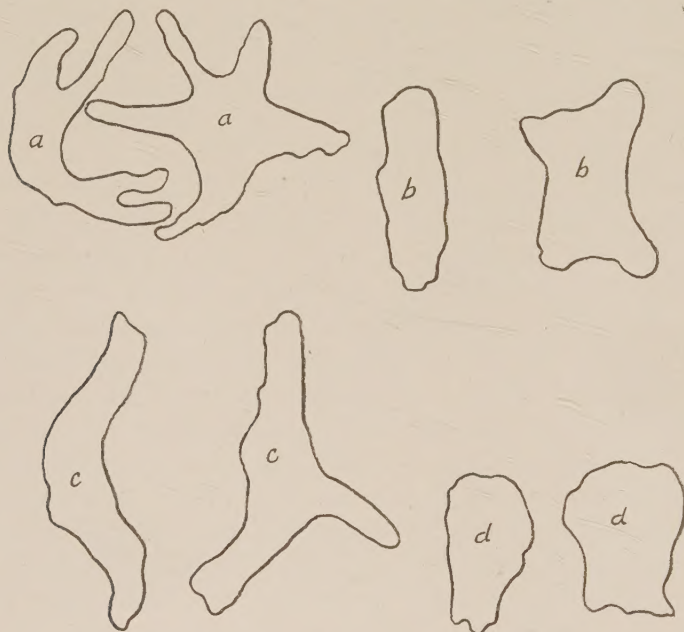


FIG. 3.—Tracings of four typical pairs of outline drawings of a plasticine model used in verifying methods of measurement; *aa*, *bb*, *cc*, *dd*, *ee* indicate the pairs; the figure to the left in each pair shows the view from above, that to the right the view from the side.

of these four masses were measured by displacement of water. The results obtained are presented in Table I and in Figure 4.

From the table it will be seen that the average percentage deviation of the calculated values from the directly measured values for volume is 5.5, indicating that if several measurements are made, and the average taken, the method is accurate to within 5.5 per cent. Since comparison between these masses and *Amoeba* might be open to question, the following experiment, using an amoeba, was performed.

Experiment 2.—An amoeba of average size was selected, and four measurements were made, each measurement being based on a series of thirty-six pairs of drawings. The results are presented in Figure 5 and in Table II.

From the table it will be seen that the four values found for the diameter are 2.97, 2.95, 3.06, and 3.00, giving an average of 2.99, with an average deviation from this value of 0.05, or 1.5 per cent, and that the corresponding values for volume are 13.18, 13.44, 14.22, and 14.14, giving an average of 13.75, with an average deviation from this value of 0.44, or 3.3 per cent. These figures represent dimensions as projected by means of the camera lucida and measured

TABLE I
MEASUREMENTS OF PLASTICINE MODELS BY THE INDIRECT METHOD
COMPARED WITH MEASUREMENTS BY DISPLACEMENT

By INDIRECT METHOD			By DISPLACEMENT	
Model No.	Diameter, Cm.	Volume, Cc.	Volume, Cc.	Percentage Difference
1.....	3.43	21.13	21.5	1.7
2.....	3.83	29.28	28.0	4.4
3.....	3.86	30.21	32.93	9.0
4.....	2.87	12.43	11.56	7.0

in centimeters on the drawings. They can be transformed into terms of actual volume or diameter in cubic millimeters or millimeters by multiplying by 0.00009 or 0.44, respectively.

From the evidence thus presented as to the accuracy of the method, it is concluded that by its use the volume of an amoeba can be ascertained to within approximately 5 per cent of its actual value.

It is immediately evident, however, that the method of plotting the axes is applicable only when the volume of the amoeba remains constant throughout the period of observation, for all observations (pairs of drawings) are treated as of equal value, without reference to the time at which they were made. If changes in volume occur, modification of this method is necessary. To provide for this contingency, two other methods of plotting were devised.

In the first, which is referred to hereafter as "selective plotting,"

a series of outlines was made as before; then from these a series was selected which when arranged in order with respect to time of

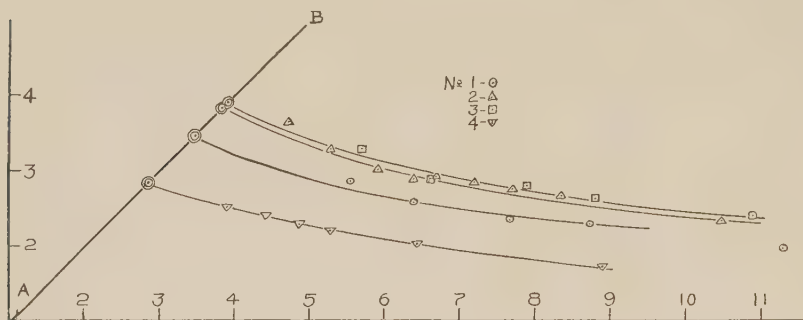


FIG. 4.—Curves obtained by plotting the axes of plasticine models molded into various amoeba-like forms. Abscissas, long axis; ordinates, short axis, of outline drawings, in centimeters: AB , locus of all points on the graph where the ordinates and abscissas are equal. Either co-ordinate of the intersection of the plotted curve with this line is approximately the diameter of the sphere equal in volume to the plasticine mass used.

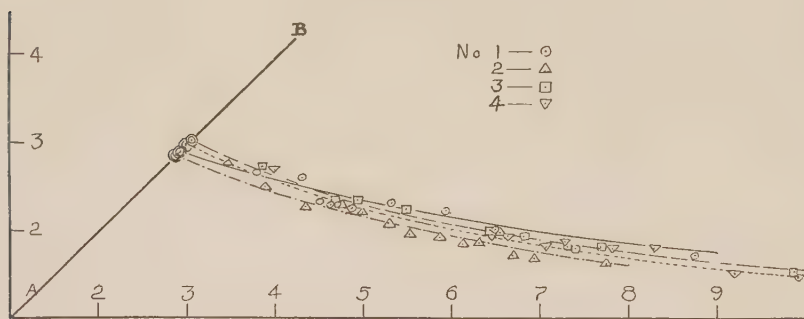


FIG. 5.—Curves obtained by plotting the axes of an amoeba. Abscissas, long axis; ordinates, short axis, of outline drawings in centimeters; AB , locus of all points on the graph where the ordinates and abscissas are equal. Either co-ordinate of the point of intersection of the plotted curve with this line is the approximate diameter of the amoeba when spherical, in arbitrary units, i.e., in centimeters as measured on the outline drawings. This diameter may be converted to the actual diameter of the amoeba in millimeters by multiplying by 0.044 and the volume calculated by means of the ordinary formula for the sphere.

making during the observation period were also arranged so that each pair had a shorter long axis than the preceding pair. From

this series the data for the axes were secured as described in the foregoing and plotted, and a curve passed as before. This method of plotting is satisfactory for ascertaining the volume at the expiration of the period of observation, but it gives little information as to the rate of change in volume, because the time intervals between the observations do not appear in the graph.

The second method, called hereafter "group plotting," overcomes this obstacle. In this a series of outlines is made as before,

TABLE II
RESULTS OF FOUR MEASUREMENTS OF ONE AMOEBA BY
THE INDIRECT METHOD*

Diameter	Volume	Deviation
2.93	13.18	.57
2.95	13.44	.31
3.06	14.22	.47
3.00	14.14	.39
Average.....	13.75	.44

*The volume is given in arbitrary units. The actual volume in cubic millimeters is obtained by multiplying by 0.00009.

and arranged in order in reference to the time each was made. Then, on the basis of the similarity of *Amoeba* to an ellipsoid of rotation, the volume of the amoeba observed is calculated from each set of outlines of the series, on the assumption that it is equal, approximately, to that of an ellipsoid with axes equal to those of the amoeba as recorded in the outlines of the series. After this, the averages obtained from successive groups of three or five of these volumes are plotted against time, and a curve is obtained that shows approximately the rate of change in the volume of the amoeba over the period of observation. In graphs given here, such curves are re-plotted in terms of percentage change (loss or gain) of the initial volume, so as to facilitate comparison. Where change from the ordinary method of plotting measurements by the indirect method was made it is indicated as noted in the foregoing.

Direct method.—In the "direct method" use was made of an apparatus consisting of a pyrex glass pipet having a capillary constriction, and an appliance for producing suction whereby an amoeba introduced into the pipet can be drawn into the constricted portion,

retained there during observation, and ejected without injury (Fig. 6). This apparatus will be referred to as the volumescope. It is used in measuring *Amoeba* as follows.

An amoeba together with sufficient fluid is placed in the mouth of the volumescope by means of a fine pipet. Then, by slightly unscrewing the cap (Fig. 6, *c*) it is sucked into the capillary part of the tube (Fig. 6, *b*). After this the volumescope is placed upon the stage of a microscope, and the amoeba is brought, by manipulation of the cap, to a predetermined part of the tube and retained there while a sketch is made of it by means of a camera lucida. Finally,

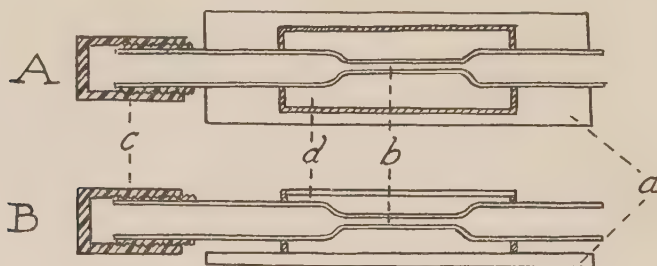


FIG. 6.—Volumescop used in ascertaining the volume of *Amoeba*. *A*, viewed from above; *B*, viewed from the side; *a*, glass microscope slide; *b*, pipet tube with capillary constriction into which the amoeba can be drawn by suction; *c*, screw cap by manipulation of which the organism is drawn into the capillary; *d*, glass-covered casing containing oil to reduce refraction and make clear observation of the amoeba while in the capillary possible.

it is ejected from the capillary portion of the tube by further manipulation of the cap. If several measurements are required, as is usual, the amoeba is left in the mouth of the volumescope until normal shape and activity are restored, after which it is again drawn into the capillary and sketched. This operation may be repeated, without injury to the amoeba, as often as desired, provided sufficient time is allowed to elapse between successive repetitions.

Since the diameter of the lumen of the capillary part of the volumescope is less than the normal diameter of the amoeba, traction into this part results in change in its form to conform to that of the lumen of the tube, i.e., the amoeba is forced to assume a cylindrical form. When in this form the volume is easily ascertained, as it is equal to $L\pi r^2$, in which L is the length of the amoeba, and r

the radius of the capillary, dimensions readily obtained from the sketches. Obviously this formula is applicable only if the ends of the amoeba take the form of parallel planes. In practice these ends are not planes but are variously curved surfaces; the measurement of length was therefore not made between the actual ends as sketched, but between points fixed by estimating the position that the ends would occupy if plane.

Change in volume is of course directly proportional to the observed change in length, provided that the same volumescope is used throughout. Only observations made with the same instrument are compared in this article.

This method necessarily involves stimulation of considerable severity, and the results presented in the following section show that such stimulation produces marked changes in volume. They, however, also show that if measurements are made in groups of five, and the interval between these groups is from ten to fifteen minutes, errors resulting from the stimulation involved in the act of measuring may be neglected.

CHANGES IN TOTAL VOLUME

THE EFFECT OF MECHANICAL STIMULATION

Lillie (1923), Lund and Logan (1925), and others assert that mechanical stimulation of cells results in alterations of permeability, and hence changes in volume. It seemed desirable to ascertain to what extent such changes in volume occur in *Amoeba*, especially so since in the application of the "direct method" of measurement the amoeba is strongly stimulated mechanically. Accordingly three experiments were performed in each of which an amoeba was subjected to a different frequency of mechanical stimulation.

Experiment 1.—An amoeba was taken from the culture and put into the volumescope, in 'culture fluid, and fifty measurements of its length, maximum and estimated, made at intervals of from two to three minutes. These two series of lengths were then averaged by tens and the resultant values compared with the average of the first ten in each series, and the differences expressed as percentage loss or gain in respect to this average, plotted against time. The maximum lengths served as a control for the error involved in esti-

mation. This was repeated with a second amoeba; the results are presented in Figure 7.

It will be seen from this figure that the first amoeba lost 8 per cent in length (proportional directly to volume) in ninety minutes, and the second 7 per cent in eighty minutes.

Experiment 2.—Ten measurements of an amoeba were made with the volumescope. Then it was transferred to a watch glass and

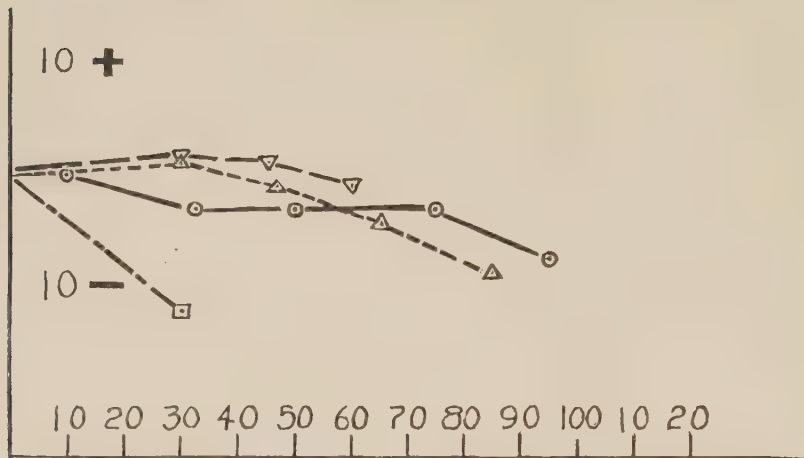


FIG. 7.—Graph showing changes in volume of *Amoeba* subjected to mechanical stimulation of various frequencies. Abscissas, period of stimulation in minutes; ordinates, losses or gains in volume as percentages of original volume; square symbols, points obtained with high-frequency stimulation; circles and triangles, points obtained with moderate-frequency stimulation; inverted triangles, points obtained with low-frequency stimulation.

agitated for thirty minutes with a glass rod. After this ten more measurements were made; the results as in the previous experiment are presented in Figure 7.

From the figure it will be seen that there was a decrease in volume of 12 per cent in thirty minutes, while in the preceding experiment there was a decrease of only 8 per cent in ninety minutes, and it is therefore concluded that the loss in volume varies directly with the frequency of the stimulation.

Experiment 3.—An amoeba was measured ten times with intervals of two or three minutes, as in the first experiment. Then the

amoeba was allowed to rest for fifteen minutes, after which the process was repeated until forty measurements had been made. The results are also presented in Figure 7.

It seems evident from these results that little if any change in volume occurred, or that if such change did occur the rest period allowed was sufficient for recovery.

The accuracy of the measurements is indicated by the fact that the average deviation of the forty measurements of the third experiment is about 2 per cent of the mean. It is therefore concluded that the method can be considered accurate to within 5 per cent or less. For this reason, and for the reason that measurements can be made with considerable ease and frequency, it is further concluded that it is preferable to the indirect method previously described in the first section, except perhaps where it is important to avoid all mechanical stimulation.

THE EFFECT OF HYPERTONIC SOLUTIONS

1. *Glycerol: indirect method.*—In using the indirect method the amoeba to be measured must be kept under observation from one to three hours. It was therefore necessary, first, to ascertain the maximum concentration to which *Amoeba* can be subjected for this length of time without injury. This was done as follows:

Ten amoebae were put into each of three watch glasses containing respectively 0.1 M, 0.2 M, and 0.3 M glycerol solution made up in culture fluid,¹ and observed at intervals over a period of three hours. It was found that the amoebae in 0.1 M and 0.2 M glycerol ceased locomotion immediately, and that at the end of three hours four in 0.3 M had disintegrated, and all the rest had become rounded and motionless. After these observations were made, all the amoebae were transferred to culture fluid. Those taken from the 0.1 M and 0.2 M solutions recovered completely in twelve hours. Those from the 0.3 M solution never recovered. The maximum concentration that can be used with this method is therefore somewhat lower than 0.3 M. The following concentrations were selected: 0.275 M, 0.2 M, and 0.1 M.

The effect on *Amoeba* of these concentrations was ascertained as

¹ Cultures used maintained by method A.

follows: An amoeba was selected from culture and two measurements (the result of calculation based on a series of sets of outlines is referred to as a measurement and a single set of two outlines is termed an observation) were made of it in culture fluid. Then 0.275 M glycerol solution was introduced into the cell, replacing the culture fluid, and a third measurement made. The observations supporting this measurement were thirty in all and covered a period of approximately two hours. After this the amoeba was put into a watch glass, washed with five changes of culture fluid, and left in culture fluid with food for twenty-four hours, after which it was again measured twice in culture fluid. It was now put into culture fluid once more and a few observations made; then the culture fluid was replaced by 0.2 M glycerol solutions, and the measurement completed, after which it was again transferred to culture fluid and washed. During the recovery period, however, it divided, preventing further measurements of this individual. A second amoeba was therefore selected, measured four times in culture fluid, and then in a 0.1 M glycerol solution.

Selective plotting was used for all observations constituting the measurements in glycerol, and the data for the amoeba in 0.1 M glycerol were also treated by group plotting. No measurement was based on less than thirty observations.

The results obtained are presented in Figures 8, 9, and 10 and in Table III.

From Figure 8 it will be seen that the curves derived from the observations in culture fluid formed two compact groups, and that in each group all the curves intersect the line *AB* at nearly the same point, indicating that there was but little variation in the results of the observations. It will also be seen that the curves obtained from the observations in the glycerol solutions all intersect the line *AB* nearer to the origin than the curves obtained from the observations in culture fluid, proving that in these solutions the amoebae decreased in volume. It will be seen from Figure 9 that this decrease varied directly with the concentration of the solutions. The absolute changes in volume can be seen in Table III, and from this it will be noted that the amoebae in 0.275 M and 0.2 M, and in 0.1 M glycerol lost 49, 29, and 19 per cent in volume respectively.

In addition to these quantitative results, it is to be noted (Fig. 8) that in the measurement in 0.2 M and in 0.1 M glycerol solution the curve obtained has a sharp break indicating a sudden drop in volume. However, in the 0.2 M solution this decrease occurred immediately after the introduction of the glycerol (the points to the right of the break are determined by calculation from the observa-

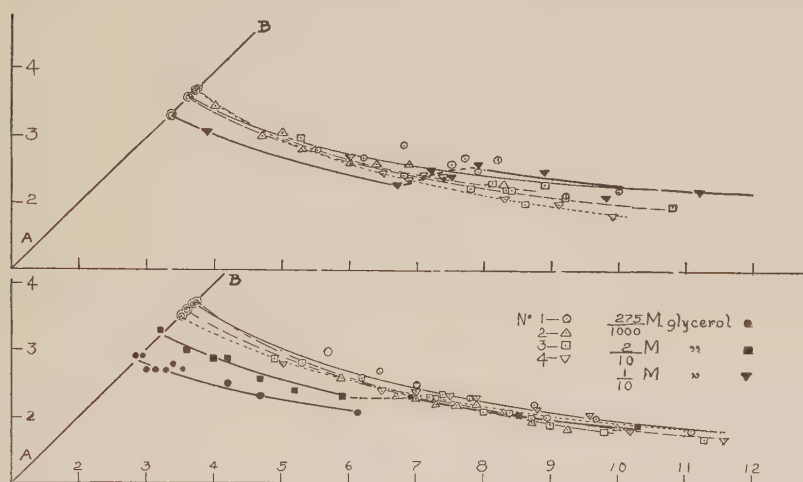


FIG. 8.—Curves plotted from axial measurements of two amoebae immersed in culture fluid, contrasted with curves obtained by plotting like measurements of the same amoebae in glycerol solutions of 0.1, 0.2, and 0.275 M concentrations. Abscissas, long axis; ordinates, short axis, as obtained from measurements of camera lucida drawings; *AB*, locus of all points on the graph where ordinates and abscissas are equal. Either co-ordinate of the point of intersection of each curve with this line is approximately the diameter of the amoeba when spherical in arbitrary units, i.e., centimeters as measured on the outline drawings. The actual diameter in millimeters can be obtained by multiplying by 0.044. Open symbols, points obtained from measurements in culture fluid; solid symbols, points obtained from measurements in glycerol solutions; solid fine line, first measurement; dot and dashed line, second measurement; dashed line, third measurement; dotted line, fourth measurement (all four in culture fluid); solid heavy lines, measurements in glycerol solutions. Note that all curves obtained for the amoebae in glycerol intersect the line *AB* nearer to the origin than any curve obtained from the same amoeba in culture fluid, proving that the amoebae decreased in volume in the glycerol solutions.

tions in culture fluid and those to the left from the observations in glycerol) while in the 0.1 M solution it did not occur until nearly two hours after the organism had been put into the glycerol, as is

shown in Figure 9. This would seem to indicate that in the more dilute solution some regulative activity in the organism prevented an immediate decrease in its volume.

2. *Lactose: indirect method.*—The effect of lactose was ascertained as follows: An amoeba was put into $\frac{1}{30}$ modified Ringer solution at pH 6.8 without food,¹ left for three hours, and then transferred to the cell and measured. After this it was returned to the Ringer solution, the cell filled with 0.15 M lactose solution, made up in $\frac{1}{30}$ Ringer solution, the amoeba replaced, and again measured. The amoeba died 114 minutes after the measurement was begun.

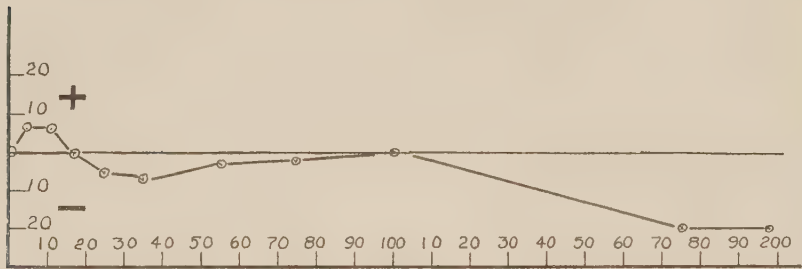


FIG. 9.—Graph showing approximate rate of change in volume in an amoeba immersed in 0.1 M glycerol solution. Abscissas, time in minutes from moment of immersion; ordinates, percentage loss or gain in volume. Note that the small fluctuations in the first part of the curve are within the experimental error, no definite change being shown until one hundred minutes have elapsed.

This was thought to be unusual, so a second amoeba was selected and subjected to the same treatment. It was in the solution 170 minutes, after which it was transferred to modified Ringer solution and found to be uninjured. The test was then repeated with another amoeba, using the direct method. Five measurements were made in $\frac{1}{30}$ Ringer solution, and then four sets of five in 0.15 M lactose solution, one thirty, one sixty, one one hundred and five, and one one hundred and eighty minutes after the amoeba had been introduced. The results of the observations on the two amoebae measured by the indirect method were treated by group plotting Figure 11.

From this figure it will be seen that the three amoebae decreased in volume 45, 48, and 55 per cent, respectively, and that the second amoeba recovered completely on transfer to the Ringer solution.

¹ Cultures used maintained by method B.

The first as noted above died, and the third was not measured after the experiment. It will also be seen that the curves obtained are all convex to the axis of abscissas, indicating that in every test the rate

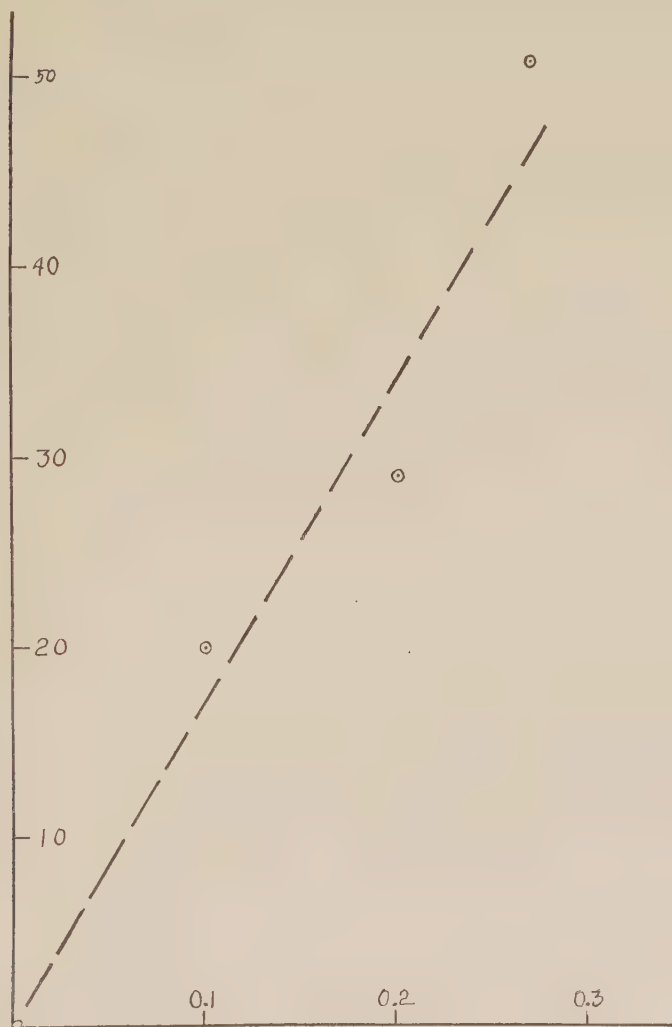


FIG. 10.—Graph showing relation between the volume of *Amoeba* and the concentration of glycerol in the surrounding medium after approximately two hours immersion at each concentration. Abscissas, concentration of glycerol in mols per liter; ordinates, percentage loss in volume.

of decrease in volume decreased with time. It is also to be noted that the percentage of decrease in volume in 0.15 M lactose is approximately the same as that in 0.275 M glycerol, indicating that the lactose solution is more effective (penetration of glycerol?).

3. *Urea*.—In ascertaining the effect of urea the procedure was essentially the same as that of the preceding experiment. A 0.15 M solution of urea made up in $\frac{1}{3}$ 6 modified Ringer solution was used. One amoeba was measured by the indirect method, and four by the direct. The results obtained by the indirect method were plotted by groups. The results obtained by both methods are presented in Figure 12.

TABLE III

CHANGES IN VOLUME IN AMOEBAE IMMERSED IN 0.275, 0.2, AND 0.1 M GLYCEROL SOLUTIONS ASCERTAINED BY USE OF THE INDIRECT METHOD*

No. Vol. in Culture Fluid	Vol. in 0.275 M Glycerol	Percentage Decrease	Vol. in 0.2 M Glycerol	Percentage Decrease	Vol. in 0.1 M Glycerol	Percentage Decrease
1. 25.5.....	12.36	49	18.15	29		
2. 25.1.....					20.3	19

* The time the amoebae were in the solution was in all cases approximately two hours. The actual volumes in cubic millimeters can be obtained from the above figures, which are in arbitrary units, by multiplying by 0.00009.

It will be seen from this figure that the amoeba measured by the indirect method decreased 40 per cent in volume, and that the four measured by the direct method decreased respectively 32, 23, 19, and 35 per cent. The period of observation was approximately two hours except in the case of the amoeba which lost 19 per cent. This amoeba, however, died after fifty minutes in the solution. It will also be noted from the figure, that the curves at first are somewhat convex toward the axis of abscissas, then nearly parallel to it, and finally concave, indicating that after a short period during which the amoebae decreased fairly rapidly in volume, the rate of decrease lessened only to become a little later more accelerated. Two hours after the experiment was concluded, all amoebae were dead, indicating that the acceleration in the decrease in volume continued until death resulted.

It is evident that there is some discrepancy between the curve obtained for the amoeba measured by the indirect method and those secured from the direct measurements, which among themselves are

very consistent. This is accounted for in part by the fact that the amoebae measured by the two methods were taken from different

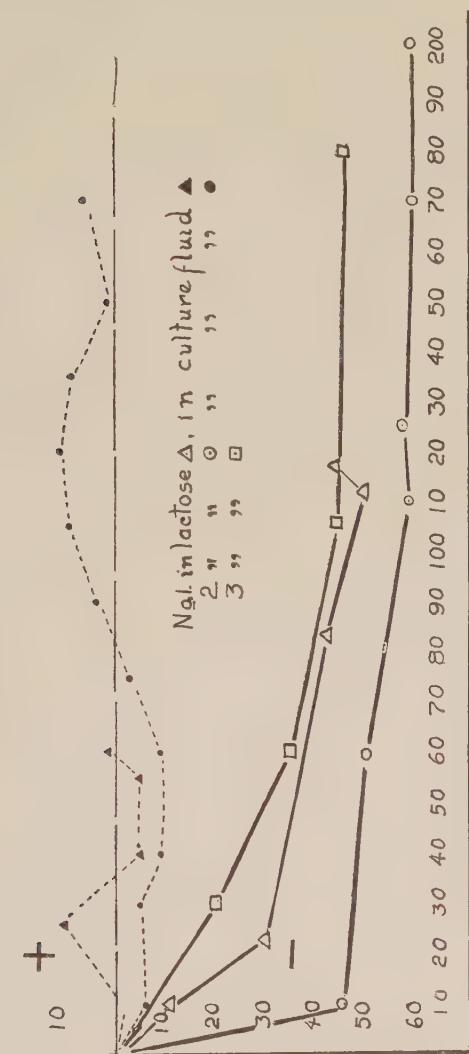


FIG. 11.—Graph showing the rate of change in volume of three amoebae immersed in 0.15 M lactose made up in modified Ringer solution. Abscissas, time in minutes from moment of immersion; ordinates, percentage loss or gain in volume; solid lines, curves for three amoebae in lactose solution; dotted lines, curves obtained from two of these amoebae in $\frac{1}{10}$ modified Ringer solution previous to immersion in the lactose. The fluctuations represented in these give some indication of the experimental error. Note that in all amoebae the rate of loss in volume decreased with time. The last two points in the dotted curve for amoeba No. 2 were obtained after recovery from immersion in lactose.

cultures, and in part by the difference in the accuracy of the methods. Moreover, the discrepancy is but quantitative and does not affect any general conclusion based on the trend of all of the curves.

By comparing Figures 11 and 12, it will be seen that the rate of decrease in volume in urea follows a different course from that in lactose.

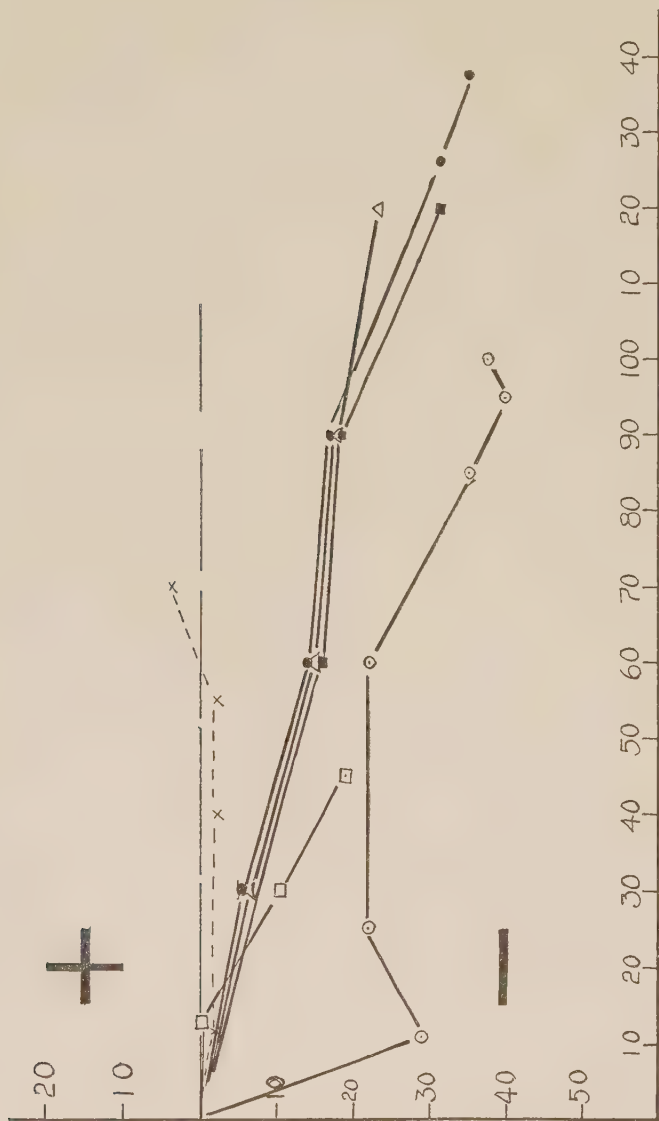


FIG. 12.—Graph showing the rate of change in volume in five amoebae immersed in 0.15 M urea made up in $\frac{1}{3}$ modified Ringer solution. Abscissas, time in minutes from immersion; ordinates, percentage loss or gain in volume; solid lines, curves for amoebae in urea solution; dotted line, curve obtained from one amoeba immersed in $\frac{1}{3}$ Ringer solution. The fluctuation in this curve shows the experimental error. The curve with circles was obtained from measurements by the indirect method, the rest by the direct method, and the amoeba used in the former was not from the same culture as those in the latter. All curves show first a decrease in volume and later an increase in this rate.

HYDROGEN-ION CONCENTRATION

Twenty-four amoebae[†] were washed in modified Ringer solution and put into modified Ringer solution at pH 7.0, left for three hours, and then measured by means of the volumescop, after which each one was put separately into a 2-cc. glass dish containing modified Ringer solution. In six of the dishes the C_H of the solution was pH 6.0, in six, pH 6.6, in six pH 7.0, and in six pH 8.0. These dishes

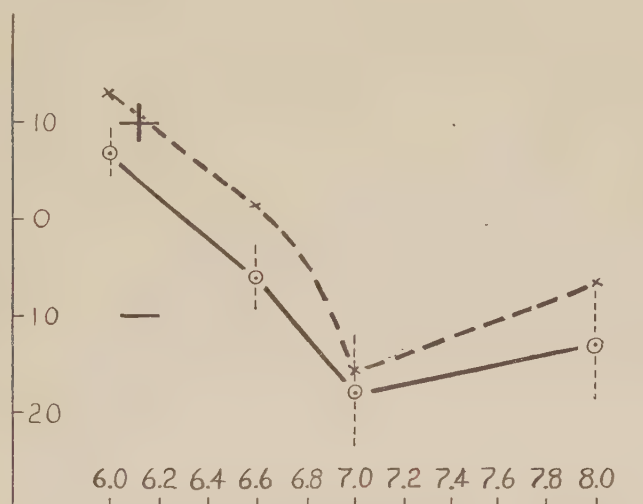


FIG. 13.—Curves showing the relation between volume in *Amoeba* and the hydrogen-ion concentration of the surrounding medium. These are based on the measurement of twenty-four amoebae immersed in $\frac{1}{6}\%$ Ringer solution at different hydrogen-ion concentrations. Abscissas, pH; ordinates, percentage loss or gain in volume; dotted lines through points show the extent of the probable error for each point.

were arranged in four equal groups in which the hydrogen-ion concentration of the contained solutions was respectively pH 6.0, 6.6, 7.0, and 8.0. Each group was then put into a 10-cc. pyrex glass dish containing enough modified Ringer solution of the same hydrogen-ion concentration as that in the 2-cc. dishes to completely immerse these small dishes. The amoebae were left in the small dishes for three hours and then again measured. The results obtained are presented in Figure 13 and in Table IV.

[†] Cultures used maintained by method C.

From the table it will be seen that there was an increase in volume in all the amoebae in the modified Ringer solution at pH 6.0,

TABLE IV
CHANGES IN VOLUME IN AMOEBAE IMMERSSED IN MODIFIED RINGER SOLUTIONS
AT DIFFERENT HYDROGEN-ION CONCENTRATIONS*

Amoeba	Initial Volume	Vol. after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Further 3 Hours in Ringer Solu- tion at pH 6.0	Percentage Change in Volume
1.....	8.0	7.5	- 6	8.3	+10
2.....	8.7	8.8	0	8.9	0
3.....	8.0	8.0	0	8.8	+10
4.....	10.4	8.8	-15	9.6	+ 9
5.....	6.3	5.6	-11	5.8	+ 4
6.....	7.3	lost			
Average....			- 6		+ 7

Amoeba	Initial Volume	Vol. after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Further 3 Hours in Ringer Solu- tion at pH 6.6	Percentage Change in Volume
7.....	6.8	6.2	- 9	5.7	- 8
8.....	8.6	8.0	- 2	7.4	- 8
9.....	8.7	8.0	- 8	7.7	- 5
10.....	9.2	9.0	- 2	9.0	0
11.....	9.1	7.4	-17	lost	
Average....			- 7		- 6

Amoeba	Initial Volume	Vol. after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Further 3 Hours in Ringer Solu- tion at pH 7.0	Percentage Change in Volume
12.....	9.2	8.0	-13	7.3	- 9
13.....	6.3	6.5	+ 3	5.5	-16
14.....	7.5	7.4	0	6.1	-18
15.....	6.3	6.2	0	4.3	-33
16.....	9.0	8.5	- 6	7.5	-11
17.....	6.8	lost			
Average....			- 3.2		-18

* Each figure is the average of five readings. To obtain actual volumes multiply figures by 0.0003. This equals the volume in cubic millimeters.

and a decrease in volume in the solutions at pH 6.6, 7.0, and 8.0. This appears certain in spite of considerable variation in the extent

TABLE IV—*Continued*

Amoeba	Initial Volume	Volume after 3 Hours in Ringer Solution at pH 7.0	Percentage Change in Volume	Vol. after a Fur her 3 Hours in Ringer Solution at pH 8.0	Percentage Change in Volume
18.....	6.5	6.9	+ 6	4.2	-30
19.....	7.0	6.7	-11	6.1	-10
20.....	8.4	8.3	0	7.6	-10
21.....	6.2	5.9	-5	5.2	-12
22.....	7.4	6.1	-18	5.9	-4
23.....	10.0	8.7	-13	8.0	-6
24.....	9.0	lost			
Average..			- 6.6		-13

of the changes in volume in individual amoebae. The curve (solid line) in Figure 13 indicates that under the conditions which obtained no change in volume occurs in modified Ringer solution at about pH 6.4, that in solutions having a higher hydrogen-ion concentration than this the volume increases, and that in those having a lower concentration it decreases.

This result does not indicate that transfer of *Amoeba* from culture fluid at pH 6.8 to modified Ringer solution at the same hydrogen-ion concentration, i.e., to the same solution without food, results in a decrease in volume, as such conclusion neglects the first transfer to modified Ringer solution at pH 7.0, which resulted in a loss in volume. To evaluate more correctly the effect on *Amoeba* of transfer from culture fluid to modified Ringer solution at the hydrogen-ion concentrations used, allowance must be made for this decrease. If this is done, by adding back the decrease in volume resulting from this initial transfer and assuming that the direct transfer would result in the same percentage changes as did the final transfers actually made, i.e., from Ringer solution at pH 7.0 to the various solutions, a curve is obtained, Figure 13 (dotted line), that indicates that if the initial transfer had been omitted the volume would have been found to be unchanged at pH 6.8 (hydrogen-ion concentration of culture) to increase in solutions more acid than this, and to decrease in those more alkaline. The greatest decrease, however, would still be found at pH 7.0. This would indicate that *Amoeba* cultured in acid solutions transferred to solutions more acid than that in which it is grown tends to gain in volume, and if trans-

ferred to solutions more alkaline to decrease in volume, but that it decreases most rapidly in a neutral solution.

Hopkins (1927) asserts that the water content of *Amoeba* is dependent upon the hydrogen-ion concentration, and (1926) that the water content is greatest at neutrality. It is evident that the foregoing confirms the first contention but disproves the last.

Assuming that the changes in volume observed are due to changes in water content the results thus far presented may be summarized as follows:

1. Mechanical stimulation of *Amoeba* results in a decrease in water content. Low frequency is less effective than high.

2. The water content of *Amoeba* decreases in hypertonic solutions of glycerol, lactose, and urea. Of these the most effective is lactose. In glycerol the percentage of decrease in volume varies directly with the concentration used. In lactose the rate of water loss decreases with time. In urea the rate of loss at first decreases and then increases. This increase probably continues until death results.

3. The water content of *Amoeba* is dependent upon the hydrogen-ion concentration of the medium. Evidence is presented tending to show that if *Amoeba* is transferred from acid culture to more acid solutions it increases in volume, and that it decreases in volume if transferred to more alkaline solutions, but that it decreases in volume most rapidly if transferred to a neutral solution.

CHANGES IN THE VOLUMETRIC RATIO BETWEEN THE PLASMAGEL AND THE PLASMASOL

It is well known that the plasmagel in *Amoeba* changes freely into plasmasol and vice versa, and that, as previously stated, these changes form one of the characteristic phenomena of locomotion (Mast, 1926*b*). Nothing is definitely known as to the cause of this transformation, but it has been surmised that changes in the water content, and in the hydrogen-ion concentration within the cell, are involved. Changes in the ratio between the plasmagel and the plasmasol have been observed, and it has been assumed that these changes depend upon environmental conditions. The experiments presented in the following pages have an important bearing upon this problem. They constitute an attempt to correlate measured changes

in the volumetric ratio between the plasmagel and the plasmasol, with definite environmental changes, and thus to ascertain some of the factors involved in formation and dissolution of the plasmagel and the plasmasol.

Two methods, A and B, were used to ascertain the volumetric ratio between the plasmagel and the plasmasol, referred to hereafter as the gel-sol ratio.

In method A the volume of the entire organism, and that of the plasmasol, was ascertained by the indirect method, group plotting being used in handling the results, and from the two curves produced, one showing the changes in the total volume and the other the changes in the volume of the plasmasol throughout the period of observation, the corresponding changes in the gel-sol ratio were readily calculated.

In method B an amoeba was put into the volutescope, placed under a microscope, and drawings made of typical sections of the organism, showing the limits of the plasmasol and the entire organism. From these drawings a series of values was obtained for the average diameter of the plasmasol and the entire amoeba, while it was in the volutescope. The squares of these values multiplied by the length of the amoeba were taken as roughly proportional to the volumes of the plasmasol and the whole amoeba, respectively, and from them the gel-sol ratio was obtained. By computing this ratio at intervals and plotting against time, a curve showing the changes in it during a period of observation is readily obtained.

By means of these two methods records were obtained of the changes in the gel-sol ratio produced by lactose, urea, and hydrogen-ion concentrations identical with those used in the previous experiments, and observations were made of the effect upon it of mechanical stimulation.

Lactose.—At the time measurement was made on the effect of lactose on the total volume in *Amoeba*, described in the preceding section under indirect method, measurements were also made of the volume of the plasmagel. From the results obtained in these two sets of measurements the changes in the gel-sol ratio during the period of observation were computed by method A. The results obtained are presented in Figure 14.

From this figure it will be seen that in one of the amoebae the gel-sol ratio increased, in fifteen minutes, from its initial value of 6.4 to a maximum of 18.0, and then decreased slowly until at the close of the experiment it reached approximately its original value; and that in the other amoeba the gel-sol ratio from an initial value of 7.4 also increased to a maximum and then decreased, but that the time taken to reach the maximum was much greater, thirty minutes, and the maximum value was only 9.3. This difference is probably connected with the fact that the first amoeba died shortly after the measurements of the plasmasol were completed. This, taken in connection with the large fluctuation in the gel-sol ratio, indicating an extreme gelation of the protoplasm, is thought to indicate an abnormal physiological state, especially since death occurred after a loss of only 49 per cent of total volume, as compared to a loss of 59 per cent in the second amoeba which suffered no injury (other than the usual rounding up that occurs in these strongly hypertonic solutions). It seems better, therefore, to consider the changes in the ratio in the second amoeba as presenting the more normal picture. By comparing these changes with the changes in total volume occurring simultaneously with them, it will be noted that during the early part of the experiment, while there was a great decrease in volume, indicating a correspondingly great decrease in water content, the gel-sol ratio increased slightly, but that during the later part of the experiment when there was but slight change in the total volume the gel-sol ratio gradually returned toward its original value.

This indicates that during the first part of the experiment water was lost from the plasmasol more rapidly than from the plasmagel, even though the plasmagel was closer to the hypertonic solution, and leads to the conclusion that the plasmagel has a greater affinity for water than the plasmasol, and acts as a semipermeable membrane between the plasmasol and the exterior. The return of the gel-sol ratio toward its initial value apparently indicates that as osmotic equilibrium is reached in respect to the exterior medium, there is a redistribution of the water content in the cell.

Urea.—The changes in the gel-sol ratio produced by immersion in urea were ascertained from measurements on two amoebae, one

measured by method A and the other by method B. The measurements by method A were secured at the same time as the measurements of the effect of 0.15 M urea on the total volume presented in the preceding section. The measurements by method B were made as described in the foregoing, in groups of five, the averages of these groups being taken. One group of five measurements was made before the immersion of the amoeba in the urea solution, and then

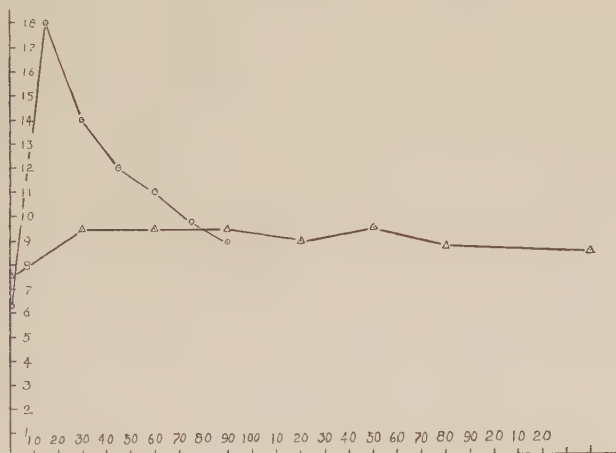


FIG. 14.—Graph showing changes in the volumetric ratio between plasmagel and plasmasol in two amoebae immersed in 0.15 M lactose made up in $\frac{1}{30}$ modified Ringer solution. Abscissas, time from immersion in minutes; ordinates, plasmagel-plasmasol ratio. The amoeba showing the very large increase and recovery of ratio died in 114 minutes after immersion. Compare these curves with Curves 1 and 2 in Figure 11, showing the changes in volume in these two amoebae during the same period.

seven more groups at five, fifteen, thirty, forty-five, sixty, seventy-five, and ninety minutes after immersion. The results obtained are presented in Figure 15.

From this figure it will be seen, on comparing the curve obtained by method A with that obtained by method B, that one indicates that there was a rise in the gel-sol ratio to a maximum during the first ten minutes, and the other does not. The fact, however, that the curve obtained by method A indicates that there was no rise does not demonstrate that a rise did not occur for no measurements were made during this time. Moreover, there is some evidence

which indicates that it actually does occur. This is presented in the following paragraph.

In lactose it has been demonstrated that there occurs a rise of the gel-sol ratio to a maximum, that this rise to a maximum is

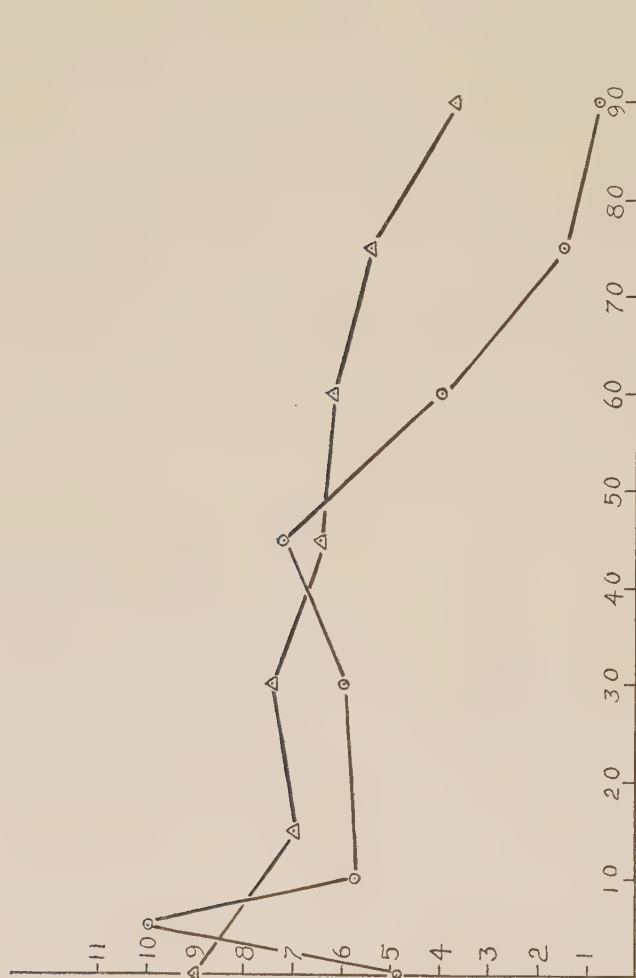


FIG. 15.—Graph showing changes in the volumetric ratio between plasmagel and plasmasol in two amoebae immersed in 0.15 M urea made up in $\frac{1}{8}$ modified Ringer solution. Abscissas, time from immersion in minutes; ordinates, plasmagel-plasmasol ratio. Note the gradual decrease in ratio after fifty minutes, also the rapid initial increase in ratio and recovery in the first ten minutes in one amoeba; compare with volume changes in the same solution shown in Figure 12.

simultaneous with a rapid loss of total volume, and that as the rate of this loss decreases, the gel-sol ratio returns toward its initial value. Now it has also been shown that in urea the rate of loss in total vol-

ume, as in lactose, is first fairly rapid, but later decreases. It seems probable, therefore, that the gel-sol ratio also follows the same course as in lactose, i.e., that it follows the course indicated in the curve obtained by method B.

It is consequently concluded that, in all probability, the actual course of the changes in total volume and in gel-sol ratio, in the urea solution, is as follows: During the first fifteen minutes or so there is a decrease in volume accompanied by an increase in the gel-sol ratio, this is followed by a recovery, partial as to total volume, and complete as to the gel-sol ratio. Then, there is a short period of slight decrease in both the total volume and the gel-sol ratio, this decrease becomes continuously accelerated until, as previously stated, death results.

If this be true, on immersion of *Amoeba* in urea solution, water is first lost from the entire organism, but more rapidly from the plasmasol than from the plasmagel, then water re-enters the amoeba for a short time, after which this swelling gives place to a further loss of water, but this time water is lost more rapidly from the plasmagel than from the plasmasol, the rate of loss accelerating until death results.

The gradually accelerated parallel decrease in the gel-sol ratio and the total volume that occurred in the later part of the experiments in which urea was used (Figs. 12 and 15) indicates strongly that changes in permeability are correlated with changes in the extent of the plasmagel. This matter will be more fully considered presently.

Hydrogen-ion concentration.—Twelve amoebae were prepared as in the experiments on the relation between hydrogen-ion concentration and total volume, and in these the changes in the gel-sol ratio were measured by method B. The results obtained are presented in Figure 16.

From this figure it will be seen that there is a very definite relation between the hydrogen-ion concentration and the gel-sol ratio, that after three hours the gel-sol ratios found at pH 6.0, 6.6, 7.0, and 8.0 were 5.25, 4.25, 1.3, and 1.37, respectively, proving that there is a decided increase in this ratio, on the acid side, and a slight increase on the alkaline side. The fact that there was a slight increase

between pH 7.0 and 8.0 indicates that continuation of the experiments in the alkaline range would show an increase in the gel-sol ratio in this range similar to that found on the acid side.

Comparison of the results obtained in this experiment with those obtained in respect to the relation between hydrogen-ion concentration and total volume, presented in the preceding section (Fig. 13) demonstrates that there is a close relation, i.e., the gel-sol ratio and

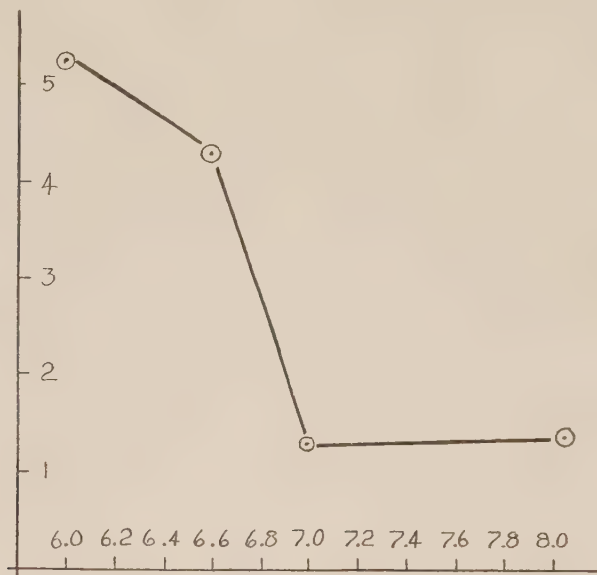


FIG. 16.—Graph showing the relation between plasmagel-plasmasol ratio and hydrogen-ion concentration. Abscissas, pH; ordinates, plasmagel-plasmasol ratio. Compare with Figure 13, showing volume changes in the same solutions.

the total volume increase or decrease *pari passu* as the hydrogen-ion concentration is altered. Thus, there is a correlation here between the thickness of the plasmagel and the water content of the cell, the two varying directly.

Mechanical stimulation.—The conclusions reached concerning the effect of mechanical stimulation on *Amoeba* are based upon observations with the volumescope on about one hundred amoebae. The results may be described as follows:

The direction of locomotion in *Amoeba*, while in the volumescope,

can be reversed by increasing the hydrostatic pressure at its anterior end. If the hydrostatic pressure at the anterior end of an amoeba moving in the capillary portion of the volumescope is increased, locomotion ceases at once, then there is a radial fissuring of the plasmagel at the posterior end. Through these fissures the plasmasol flows to form numerous fine pseudopodia which coalesce about the remaining masses of the old plasmagel. After this occurs the old gel rapidly disintegrates and a new anterior end is formed from the plasmagel of the coalesced pseudopodia. This then bulges out and locomotion begins in the new direction. On the contrary, if the hydrostatic pressure is increased at the posterior end, it simply results in a more rapid bulging of the anterior end and an increased rate of locomotion.

If reversal of the direction of locomotion has been induced as described in the foregoing, and an attempt is immediately made to again reverse the direction of travel, no fissuring occurs at the newly formed posterior end. There is simply a thinning of the plasmagel under the pressure, and a reversal of streaming, the newly formed plasmagel reverting to the sol state over a large part of the surface free in the tube, and then as the exterior membrane (plasmalemma) bulges forward locomotion begins. However, if a few minutes intervene before a second reversal is attempted, the fissuring and the other associated processes occur as described.

These facts indicate that there is a decided difference in rigidity between newly formed and old plasmagel, and that this difference is related to the age of the gel, the old gel being more rigid than the new. This tends strongly to support the contention of Mast (1926) that such a difference exists.

In these observations it was also noted that if the reversal is repeated at frequent intervals, i.e., approximately once a minute, for twenty or thirty minutes, reconstruction of the plasmagel becomes progressively more and more sluggish and its dissolution at each reversal more extensive. In fact, it gradually comes to involve the entire plasmagel, locomotion ceases as a normal process, and the entire mass within the plasmalemma becomes plasmasol. From this it is concluded that mechanical stimulation decreases the gel-sol ratio. Taking this fact into consideration together with the fact, as

demonstrated in the previous section that mechanical stimulation causes a loss in volume which increases as the stimulation is increased, it being easy to cause a loss of 12 per cent, it is further concluded that breaking down of the plasmagel renders *Amoeba* more permeable, and results in a decrease in water content and in volume.

DISCUSSION

In the preceding pages, it has been demonstrated that *Amoeba*, on immersion in a hypertonic solution of lactose or urea, decreases in volume, but that in lactose the rate of decrease decreases with time, while in urea it increases. It has also been demonstrated that in lactose solution the gel-sol ratio at first increases slightly and then returns toward its initial value after which it remains practically constant, while in the urea solution this ratio, after undergoing a similar change, does not remain constant but continues to decrease.

These facts indicate that in both solutions water is abstracted from the amoeba, first more rapidly from the plasmasol than from the plasmagel and then vice versa; but that in lactose the decrease in water content of the entire organism and of the plasmasol tends to cease, while in urea it is accelerated. This leads to the conclusion that some factor becomes active in abstraction in urea that does not appear in lactose.

Evidence has been presented indicating that if *Amoeba* is transferred from a slightly acid culture medium to $\frac{1}{10}$ modified Ringer solution at different hydrogen-ion concentrations, it increases in volume in solutions that are more acid than the culture, and decreases in those that are more alkaline, and that the greatest decrease occurs in the neutral solution. It has been demonstrated that under these conditions the gel-sol ratio is lowest at neutrality and higher in either direction.

This indicates that the water content changes *pari passu* with the changes in the gel-sol ratio as it does in the latter part of the experiments in urea.

Finally it has been observed that when *Amoeba* is subjected to mechanical stimulation it decreases in volume, and that likewise the gel-sol ratio decreases.

It seems evident from the foregoing that the water content in

Amoeba varies sometimes directly and sometimes inversely with the gel-sol ratio. The question arises as to how this difference can be explained.

To attempt an explanation it is necessary first to examine the experimental results to see if they indicate the mechanism involved. It will be noted that the water content varies directly with the gel-sol ratio when *Amoeba* is subjected to different hydrogen-ion concentrations, and that the variation in both water content and in the ratio yields curves (Figs. 13 and 16) that are similar to the curves obtained for the changes in water content of an amphoteric hydrogel, such as gelatin gel subjected to like conditions. The changes in water content that occur upon mechanical stimulation, taken in connection with the accompanying changes in the gel-sol ratio, appear to indicate that the plasmagel serves to a large extent in retaining the water content of the amoeba, since its destruction by stimulation is followed by reduction in volume, i.e., in water content. Further, the initial portions of the curves obtained in urea and lactose, since they indicate that water is lost in hypertonic solutions more rapidly from the plasmasol than from the plasmagel, appear to indicate that in such retention of water the plasmagel acts as a semipermeable membrane with respect to the plasmasol. Finally the change in the curvature in the latter part of the curve showing the results in urea indicates that urea has upon the plasmagel a dispersive effect possibly similar to its effect upon gelatin gels (Bechold, 1905, p. 55).

Taking all these facts, and the fact that the activity of the plasmagel appears to be minimum at neutrality, as shown in Figs. 13 and 15, it appears reasonable to assume that the permeability in *Amoeba* and therefore its water content is to a large extent controlled by the plasmagel, and that the factor exercising this control is an amphoteric colloid, probably a hydrogel, within the plasmagel, which has an isoelectric point at about pH 7.0 and is affected by urea like gelatin.

On the basis of this assumption the following will obtain:

Amoeba immersed in lactose will lose in volume, owing to the osmotic pressure of the medium. However, the plasmagel will lose water less readily than the other cell constituents, since the colloid

in the plasmagel being hydrated (i.e., holding water by adsorption on its micellae, and hence not free to move as liquid) cannot part readily with its water content. As equilibrium is reached equalization of the normal relation will be restored, or nearly so provided that the loss of water of hydration from the colloid is slower than the loss of water from the cell due to osmotic pressure.

Amoeba immersed in hypertonic urea solution will at first be affected by the osmotic pressure just as in lactose, but due to the effect of the urea, the colloid after a short time will be broken down. Then the cell will become more permeable and this will result in a simultaneous decrease in the gel-sol ratio and in the volume, for the gel-sol ratio is controlled by the condition of the colloid, and as this is dispersed the ratio must change, and likewise since the condition of the colloid controls the permeability its dispersion will entail an increase in permeability with loss of salts and water content.

Amoeba immersed in hydrogen-ion concentrations more acid or more basic than the culture fluid will alter in volume just as does a gelatin gel under similar conditions, and like such a gel will have least volume at its isoelectric point. The gel-sol ratio, since it is dependent upon the hydration condition of the protein here assumed, will likewise alter, in fact the changes in volume described in the preceding are the changes in volume undergone by the plasmagel as distinguished from the plasmasol. That is, the changes in gel-sol ratio and volume will be due to increase or decrease of the hydration of the colloid at the different hydrogen-ion concentrations, with their consequent effects.

Mechanical stimulation will result in disintegration of the hydrogel and consequent loss of water content, since it is assumed that integrity of this gel is in large part responsible for the semipermeability of the cell.

In regard to such injury by mechanical stimulus, it might be suggestive to consider the possibility of the colloid being present within the plasmagel in the form of a film structure such as described by Mast (1926a), i.e., as filling the interstices between the included vacuoles and granules. If this obtains mechanical stimulation might cause rupture of the films, thus lessening the thickness of the plasmagel as a whole. Also it is pertinent to note that Lepeschkin (1926) asserts that mechanical stimulation of proto-

plasmic colloids results in coagulation, and that Gurchot (1926) contends that partial coagulation of membranes will render them more permeable. Hence, applied to the plasmagel, admittedly colloid in structure, the findings of these two investigators would lead to the expectation that mechanical stimulation would lead to an increase of permeability in any cell membrane, with consequent loss in volume.

The question now arises as to what bearing the demonstrated facts, taken in connection with the hypotheses advanced in explanation of them, have upon the problem of the gel-sol transformation in locomotion.

As stated in the foregoing the colloid of the plasmagel was assumed to be of importance in respect to the permeability of *Amoeba*, and it was also implicitly assumed that its thickness is a measure of its effectiveness. It has been definitely shown, except under conditions in which osmotic pressure is presumably the sole agent, that when a change in volume occurs in *Amoeba* which requires for its explanation the postulation of changes in permeability, the water content changes directly with the gel-sol ratio. This indicates, if the postulated colloid exists, that hydration of a protein or similar substance is a factor in the gel-sol transformation, for it is obvious that this colloid must be present both in the plasmagel and the plasmasol, and increase of the plasmagel relative to the plasmasol, with increasing water content, must mean an increase in extension of the hydrated colloid in the plasmagel with increase of hydration of the cell, and a decrease of this with dehydration. These experiments therefore are in line with the conclusions of Pantin (1925) that a process of hydration and dehydration occurs as an integral part of the process of locomotion, since it would appear from the foregoing that it must be considered that the plasmagel is formed from the plasmasol by imbibition of water, or absorption, of it, on the particles of the colloid.

The findings also support Mast's contention (1926b) that the plasmagel plays the part of a semipermeable membrane with respect to the plasmasol.¹

¹ The writer desires to express his sincere thanks to Professor S. O. Mast, who suggested the problem and under whose guidance the work was done, for his continued

SUMMARY

1. Two methods are given for the measurement of volume in *Amoeba*. In one the volume is ascertained by calculation from measurements on outline drawings of *Amoeba* as seen from above and from the side. In the other it is ascertained by measurement after the amoeba has been caused to assume the form of a cylinder of known diameter. The methods are accurate to somewhat less than 10 and 5 per cent, respectively.

2. *Amoeba* immersed in hypertonic solutions of glycerol, lactose, or urea decreases in volume. In glycerol solutions the volume varies directly with concentration, over the range employed. In lactose and glycerol the rate of decrease in volume diminishes with time until the volume becomes constant. *Amoeba* transferred from $\frac{1}{3}0$ modified Ringer solution at pH 6.8 to 0.15 M lactose solution of the same C_{π} loses approximately 50 per cent in volume. In urea the rate of decrease in volume first diminishes and then increases with time until death. In glycerol and lactose the decrease in volume is probably the result of loss of water by diffusion, owing to the hypertonicity of the medium. In urea it is probable that while this obtains at first there is later a loss of water owing to increase in permeability brought about by the action of the urea on the protoplasm.

3. Mechanical stimulation breaks down the plasmagel structure in *Amoeba*, and this is accompanied by a decrease in water content. High frequency stimulation is more effective than low.

4. *Amoeba* transferred from a culture at pH 6.8 to a series of $\frac{1}{3}0$ modified Ringer solutions of different hydrogen-ion concentrations loses water most rapidly at neutrality, and gradually less rapidly in either direction. On the acid side the loss is replaced by a gain in solutions more acid than pH 6.4. It is probable that *Amoeba* if transferred from a slightly acid culture medium to a more acid medium will gain in volume, and that if it is transferred to more alkaline media it will lose.

5. In hypertonic solutions of urea the volumetric ratio between the plasmagel and the plasmasol decreases after a sharp initial increase. This decrease is accelerated with time. The initial change

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is probably due to a disturbance of equilibrium across the plasmagel. The final decrease is probably due to the action of the urea on a colloid structure in the plasmagel, similar to its action upon gelatin gels, leading to an increase in the permeability of the cell which results in entrance of urea and either destruction of the colloid structure or interference with the plasmagel-plasmasol transformation.

6. In hypertonic solutions of lactose the volumetric ratio between plasmagel and plasmasol at first increases and then returns toward its initial value. The initial change is probably due to disturbance of equilibrium across the plasmagel which probably holds part of the water contained in a bound condition, and therefore although in fact more hydrated than, or at least equally hydrated with, the plasmasol, acts as if less hydrated, the plasmasol water being free, being more readily abstracted from the cell. The recovery later is due to transformation of plasmagel to plasmasol or redistribution of water content within the cell, which restores the equilibrium between free and bound water (i.e., water adsorbed and forming an integral part of the colloid structure of the plasmagel).

7. The volumetric ratio between plasmagel and plasmasol is dependent upon the hydrogen-ion concentration of the surrounding medium. The ratio varies from 5.25 at pH 6.6 to 1.4 at pH 7.0 and 8.0. There is probably a tendency to an increase in this ratio in alkaline solutions as compared with neutral solutions.

8. The transformation of plasmasol to plasmagel in *Amoeba* is apparently the result of the formation within the plasmasol of a colloid interprotoplasmic structure, and the transformation of plasmagel to plasmasol the result of the dissolution of this structure. It is postulated that the formation of this structure is the result of hydration of micellae composed of an amphoteric substance with an iso-electric point at about pH 7.0, and its dissolution is due to their dehydration, which results in flocculation.

9. Since increase and decrease in water content vary directly with the thickness of the plasmagel, except under conditions in which osmotic pressure is probably the sole agent effecting the transfer of water through the cell surface, it appears that in *Amoeba* changes in permeability are probably functions of the plasmagel.

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VITA

Harold William Chalkley was born in London, England, March 19, 1887. He received his elementary education in the public schools of Lawrence, Kansas. He attended the University of Kansas during the years 1906-7. He received the degree of Bachelor of Science with Honors from the Mississippi Agricultural College in 1924. He received the Degree of Master of Arts from the Johns Hopkins University in 1926.

